The Effect Of Burns
On Living Tissues

by

Ellen Butler Dunn

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Thesis

THE EFFECT OF BURNS
ON LIVING TISSUES

by

Ellen Butler Dunn
(A.B., Wellesley College, 1944)
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Approved

by

First Reader ....Brenton R. Lutz........
Professor of Biology

Second Reader ....Genevieve Young........
Assistant Professor of Biology

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THE EFFECT OF BURNS
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INTRODUCTION

Ever since man first came in contact with fire, the treatment of burns has been a problem, one which has become less important with the advent of electricity, perhaps, but which is still important. The treatments have evolved quite normally by trial and error, but this is a rather lengthy and uncertain method. Consequently, efforts have been made to determine the exact sequence of events when tissue is burned, so that more intelligent treatments can be applied.

Since any injury to living tissue affects the organism as a whole, slightly or greatly, depending on the extent of the injury, it would be very difficult to include in this thesis all the effects of a burn, even assuming that they could be determined. Therefore, only those effects occurring

immediately within and surrounding the burned area will be considered. All others, such as shock, for example, will be excluded except where they serve as evidence for an immediate effect.

The changes in tissues brought about by a burn seem to fall naturally into three phases: the initial destruction, the subsequent inflammation, and the final healing. It is under these headings that the review of the literature will be organized.

The experimental work included in this thesis is concerned with the effect of burning on a small area of an unstained retrolingual membrane of the tongue of Rana pipiens, commonly known as the leopard frog. The work was done on the living frog, and observed daily. The observations are of the destruction, inflammation, and healing of the tissues in the damaged area, with figures and tables to illustrate the work.

The author wishes to express her deep appreciation to Professor Brenton R. Lutz for his help and for many suggestions concerning the research, and to both Professor Lutz and Assistant Professor Genevieve Young for their valuable comments and assistance in organizing and presenting the material.

REVIEW OF THE LITERATURE

Destruction of Tissues.

Most laymen will agree that a severe burn causes destruction of tissues, and would offer as proof the hole left by the burn or the healing required to cover the raw wound. As a matter of fact, it is virtually self-evident that destruction occurs; in more scientific terms, it may be defined as irreversible damage to tissue resulting in necrosis.

In scientific literature, evidence that a sufficiently severe burn causes tissue destruction has been presented in papers by Best and McHenry (1931); Strassmann (1933); Bonciu (1935); Dann, Glucksmann, and Tansley (1941); Hughes and Dann (1941); Elman, Cox, Lischer, and Muller (1942); Leach, Peters, and Rossiter (1943); Evans and Hoover (1943); Prinzemetal, Hechter, Margoles, and Feigen (1944); Ham (1944, 1944a); Hara (1929); Shen and Haw (1943); MacDonald, Levenson, Davidson, Tagnon, and Taylor (1944); and Harkins (1945).

The first listed of these authorities, Best and McHenry (1931), offered indirect evidence of tissue destruction in their finding that extensive burns cause an ensuing state of shock, like that produced by large doses of histamine. They also stated that the concentration of the blood increases. The former effect would indicate destruction of

tissues (Findlay, 1928); and the latter phenomenon would result from injury to the blood vessel walls, causing increased permeability, the outflow of fluids producing the increased concentration of the blood. (This aspect will be more fully discussed under inflammation.)

Strassmann (1933) passed a direct current from a 220-volt source through living experimental animals and corpses. He found that holes appeared in the dry skin at the anode, a certain indication of tissue destruction.

Bonciu (1935) offered both direct and indirect evidence of necrosis. Using guinea pigs and burning their internal organs with a gas flame to produce first, second, and third degree burns, he reported finding desquamated epithelial cells and red blood cells in the lumina of the kidney tubules and marked lesions in the cortex. Hemorrhages into Bowman's capsule were found, caused quite probably by damage to the capillary walls of the glomeruli. This capillary damage was sometimes further substantiated by the appearance of an albuminous exudate both in Bowman's capsules and the lumina of the tubules.

Dann, Glucksmann, and Tansley (1941) give more direct evidence of tissue destruction. Working with 9 to 18 month old rats, under anesthesia, they burned each animal on the back with an electric soldering iron. They then killed the

rats on successive days up to two months after infliction of the burn, stained and made sections of the burned areas. On microscopic examination, Dann and his co-workers reported finding that the damage done to the tissues is inversely proportional to the distance of the tissues being examined from the place of burning; those immediately under the soldering iron are injured by direct heat and the more distant tissues by conducted heat. The tissues killed by the direct heat become necrotic and drop out of the wound on the tenth day.

In further work using the same methods, Hughes and Dann (1941) found that dilatation of capillaries immediately followed a burn, particularly in the superficial capillaries of the skin, which were normally quite fine. With damage to the underlying muscle tissue, the condition was much exaggerated. (This again would indicate injury to the vessel walls.) They also found that some vessels were destroyed, and of those which were not, the lumens were filled with clotted blood.

Elman, Cox, Lischer and Muller (1942) classified burns according to the amount of destruction. They reported that a low temperature burn of short duration results in edema (injury to the capillary walls, as evidenced by increased capillary permeability.) A medium temperature burn of longer duration results in wet necrosis, and a high temperature burn

of still longer duration produces dry necrosis.

Further results substantiating tissue destruction were presented by Leach, Peters, and Rossiter (1943). Guinea pigs and rats were shaved, anesthetized, and exposed to graded temperatures of 45° - 80° C. from 10 seconds to 6 to 10 minutes. In burning at 47° C. for times up to 6 minutes, no destruction occurred, the temperature not being high enough to cause damage. At 50° - 55° C. for one minute, damage always occurred. At 60° - 65° C. the epidermis was so damaged that it peeled off and marked edema resulted, indicating injury to the capillary walls. With these milder burns, cellular disintegration became noticeable, and with the more intense burns, at temperatures of 70° - 80° C. for 10 to 20 seconds, severe scabbing occurred, indicating rather extensive damage. Coagulation was also observed in the most central portion of the burn, with areas around the center containing tissue changes like those of the less intense burns.

Prinzmetal, Hechter, Margoles, and Feigen (1943), using rats, produced what they considered a mild burn on a single hind limb by a temperature of 75° C. applied for 10 seconds. They obtained marked edema, which would again indicate either damage or destruction of the capillaries, or both. Upon infliction of what was classified as a severe burn, that produced by a temperature of 100° C. for 2 to 3 minutes, the

animals died in shock, but there was no visible edema. This seems to corroborate the work of Elman and his co-workers (1942) reporting dry necrosis at high temperatures, and that of Leach, Peters and Rossiter (1943) reporting coagulation due to heat with an intense burn. The probable occurrence of coagulation in the above case is indicated by the lack of edema; and coagulation of protein would certainly be an irreversible change and an example of destruction. The death of the test animals in shock would seem to indicate, in addition, that considerable tissue destruction took place (Findlay, 1928).

In the work by Ham (1944 and 1944a), coagulation of the superficial vessels of the skin was found in severe second degree burns. Plasma loss occurred in capillaries directly under this area, and in the region of the hair follicles the capillaries were dilated and congested, and leaked plasma. In severe third degree burns the capillaries in the subcutaneous region of the skin were so damaged by the heat that they behaved like the superficial capillaries of the skin in a severe second degree burn. Evans and Hoover (1943) also reported that third degree burns cause increased capillary permeability.

Evidently not only the capillary walls but the contents of the vessels are damaged in burning, for Hara (1929)

reported that hemoglobinuria of both human and rabbit blood results from a burn. Shen and Haw (1943) reported that, in 40 patients with second and third degree burns, five showed a marked increase in the fragility of the erythrocytes and that fragmentation and acid urine resulted in eleven patients in the first 48 hours following the burn. This is again a definite indication of necrosis. MacDonald and his co-workers (1944) did studies on patients with third degree burns and found that all had hemoglobinemia and also thrombocytopenia, and that the thrombocyte count did not return to normal for four to nine days following the burn.

Destruction of tissues is, then, a well established result accompanying a sufficiently severe burn; it includes all tissues which the burn affects, either by direct or conducted heat. Harkins (1945), in his paper The Present Status of the Problem of Thermal Burns, states that necrosis always results from a deep burn, owing to tissue destruction. Evidences of such destruction are coagulation of the tissues, edema due to increased capillary permeability resulting from damage to the walls, increased fragility of the erythrocytes, and necrosis, with the subsequent disintegration of the tissues.

Inflammation and Infection of Burned Tissue.

Inflammation is usually defined as the appearance in tissue of heat, redness, and edema or swelling. Scientific literature in general explains that the heat and redness are caused by dilatation of the capillaries of the inflamed area, and that the edema is produced by increased capillary permeability, with its resultant increase in tissue fluid. Menkin (1940), however, reported that inflammation involves not only an increase of tissue fluid in the area, but also the formation in some cases of thrombi and fibrinous networks which wall off the lymphatics, and the certain migration first of leucocytes and later of macrophages into the inflamed area. Menkin's analysis does not refer specifically to burned tissue, but to tissue inflamed by any cause.

In 1937, Menkin had postulated the existence of a substance which he called leucotaxin, which effected increased capillary permeability and tissue fluid, and also caused migration of leucocytes into the inflamed area. By 1938 he had isolated a crystalline nitrogenous substance from inflammatory exudates, which, when placed in a non-inflamed area, caused the typical symptoms of inflammation. Menkin, Kadish, and Summers (1942) recovered from human empyema fluids an active globulin fraction which, when given to dogs, produced a rise in the number of leucocytes circulating in the bloodstream.

Direct evidence of increased capillary permeability was reported by Menkin and Menkin (1930). Injecting trypan blue into the capillaries of the peritoneal cavity of frogs, they found twice as great a decrease in concentration of the dye to occur in capillaries of an inflamed area as that in an uninflamed area.

In the case of a burn, in which Menkin (1940) maintained inflammation is always present, the picture is further complicated by the possibility of initial damage to the capillary walls from the intense heat. It is not necessary to wait until infection has set in or an irritating substance has been applied to get the classic inflammation pattern.

Direct and indirect evidence of increased capillary permeability in the immediate vicinity following a burn is given by Underhill and co-workers (1930); Underhill (1930-31); Best and McHenry (1931); Beard and Blalock (1931); McIver (1933); Clark and Clark (1933); Ely (1943); Netsky and Leiter (1943); Cope and Moore (1944); and Harkins (1945).

Underhill, Kapsinow, and Fisk (1930) shaved rabbits, anesthetized them, and burned an area of skin by moving an electric flat iron over it. They reported dilation of the capillaries, followed by packing of erythrocytes in the capillaries, and finally by extravasation of the erythrocytes through the capillary walls. Using the same method, Underhill, Kapsinow, and Fisk (1930a) injected organic dyes,

methylene blue, Trypan blue, or phenolsulphonephthalein into burned rabbits. The dyes left the blood stream in the burned region but not in the unburned regions, for the dyes were recovered only in the edema fluid of the burned area. There was an eventual reabsorption of the fluid, which was interpreted as a result of repair of the capillary wall and reëstablishment of osmotic and hydrostatic pressures. Underhill and Fisk (1930) found the edema fluid resulting from a burn to be similar to serum and blood plasma. From their analysis, it is difficult to tell whether they meant serum or blood plasma, for they appear to use the two terms synonymously; this is incorrect, serum being blood plasma minus fibrinogen. Plasma was probably meant, since both Beard and Blalock (1931) and McIver (1933) reported that the edema fluid was like plasma. In either case, increased permeability of the capillary walls is indicated by the appearance of the fluid.

In further work by Underhill, Fisk, and Kapsinow (1930) a greater moisture content is reported to exist in both burned skin and muscle than in unburned skin and muscle. Again this would indicate increased capillary permeability. Further substantiation is found in the work of Ely (1943), who anesthetized rats and burned their rear legs over a spot 1.25 inches in diameter, using a hot iron (400° C.) for 5 seconds. Ely reported greater moisture content in the burned

leg.

Best and McHenry (1931) observed an increase in blood concentration following burns, due to increased capillary permeability and the filtering out of the plasma-like fluid. Burning rabbits (which do not have functional sweat glands), Underhill (1930-31) obtained subcutaneous blisters, the fluid of which collected in finger-shaped pockets which were easy to reach and drain off the fluid for examination. The larger the area of body burned, the greater was the amount of fluid which appeared in these blisters, and the higher became the concentration of the blood.

Clark and Clark (1933) reported that inflammation results from overheating the tissues in the transparent chamber in a rabbit's ear. They found "free fluid" in the tissue spaces outside the vessels, and even observed in one of the vessels a minute hole, which gradually disappeared.

Perhaps two of the most significant experiments were made by Netsky and Leiter (1943) and Cope and Moore (1944). The former injected horse serum protein into dogs; in unburned dogs the horse serum was picked up much more slowly by the lymphatics than in burned dogs. Cope and Moore used inorganic and colloidal compounds tagged with radioactive dyes, and found that in burned animals the colloids passed through the capillary walls as rapidly as did the inorganic compounds in

the unburned animals.

Whether walling off the lymphatics, the second element in Menkin's description of inflammation, is caused by the burn alone is unknown and somewhat doubtful. Menkin observed the walling off the lymphatics in inflammation caused by infections, not in inflammation caused by burns. To date, the writer has been unable to locate any research in which such walling off has been caused directly, or even indirectly, by heat damage; but such a relation is quite possible. Dann, Glucksmann, and Tansley (1941) observed the infection of 33% of their experimental burns. Harkins (1945) also reported that infections with burns are quite common, and extremely difficult to prevent. An explanation for the prevalence of infection was offered by Findlay (1928), who reported that an area inflamed by any cause was more likely to become infected than an uninflamed area because of its increased capillary permeability (permitting organisms to leave the blood stream more easily and pass out into the tissues). It is also known that the reverse effect of drying is harmful if not lethal to most bacteria. Further research may definitely establish that walling off the lymphatics can indeed result indirectly from burns, due to the susceptibility of the raw inflamed area to infection.

When infection of a burn does occur, healing of the

area is slowed, and the course of the healing is irregular (Dann, Glucksmann, and Tansley, in 1941; and Glenn, 1944). This will be discussed further under the heading of "Regeneration".

The pattern of vascular response remains the same in inflamed, infected areas as it is in inflamed, uninfected areas (Menkin, 1940). Nakahara (1925) injected oil into the peritoneal cavities of white mice, and observed macrophages appearing in the region and ingesting the oil. Clark, Clark, and Rex (1936) made observations by transillumination of the tails of amphibian larvae and of rabbits' ears, through the transparent chamber. They found that the first cells which appear in inflamed areas, in both amphibians and mammals, are polymorphonuclear leucocytes. Menkin in 1940 reported the sequence of appearance of cells to be: first, polymorphonuclear leucocytes and erythrocytes; and second, macrophages. He discovered that the pH number of an inflamed area is initially 7.2 to 7.4, and that it gradually decreases to approximately 6.8. The first pH range favors the existence of leucocytes, and the second, more acidic state favors macrophages. An even greater acidity of the membrane results in suppuration.
Jones (1945) reported the same cellular sequence as Menkin (1940): polymorphonuclear leucocytes and erythrocytes, followed by macrophages.

The classic heat, swelling, and edema pattern of inflammation, then, may be elicited not only by infections or irritating substances, but also directly by burns, and results from heat damage to the capillary wall, which increases capillary permeability and causes loss of plasma from the vessel. In addition, a burn, acting indirectly through infection, may possibly cause the walling off of lymphatics, and produces a slow and somewhat irregular healing process. It is also to be noted that the same cellular sequence that is present in inflammation is also present in burns.

Healing of Burned Tissue and the Influence of Infection.

Tissue injured by mechanical, chemical, or thermal means will heal, provided the injury is not so severe as to require grafting. Regeneration of epithelial and connective tissues, muscle fibers, capillaries, and lymphatics in wounds made by means other than chemical or thermal is discussed in the work of Sandison (1928, 1928a), Clark and Clark (1932, 1933, 1937, 1939), Arey (1932), Stearns (1940), and Dann, Glucksmann, and Tansley (1941).

Sandison (1928, 1928a) introduced a transparent chamber into a rabbit's ear. Since it was necessary to cut a section out of the ear, that is, to inflict an actual wound, in order to insert the chamber, regeneration of the tissue subsequently occurred. A fibrinous network formed immediately after the operation, and erythrocytes and polymorphonuclear leucocytes (the inflammation pattern) appeared within the next 24 hours, followed by macrophages, with an attendant increase in the number of leucocytes. The fibrinous network became even more extensive, consisting of both light and heavy strands. By the third day, the fibrinous network filled the entire area, and hemorrhages marked the advancing area of capillary growth. The tips of the capillaries were bulbous and, under gentle pressure, extruded erythrocytes. In several days, pressure would no longer cause this extrusion, indicating more complete

formation of the walls. Gradually muscle layers formed around some of the capillaries, and they developed into arterioles and arteries.

In his second paper (1928a), Sandison reported in more detail on the development of blood vessels. This process is characterized by the formation of the fibrinous network, then the migration of fibroblasts and other cells over the network, followed by the development of connective tissue fibers in parallel masses, and the appearance of a heavy area of extravasated erythrocytes on the advancing border of the growing capillaries. Frequently, the dense mass of newly formed connective tissue fibers and the extravasated erythrocytes obscured the development of the blood vessels at this point, but sufficient successful observations were made to reveal the pattern of blood vessel formation. At first, solid threads developed from the preëxisting patent vessels. The threads varied in length and tended to be quite fine, particularly at the free tips. They might connect two capillaries or merely branch out from the vessel itself. A lumen, narrow at first but later widening, developed in each thread. Blood was forced out into the lumina, and might be extruded from the tips. Finally pulsation and then actual circulation appeared, with the establishment of patency. There was first a great profusion of these newly formed patent capillaries, which lengthened and gradually developed into arterioles or venules

and eventually, in some cases, into veins and arteries, by the accumulation of muscle layers around the vessels and changes in the diameters of the lumens. Some vessels remained capillaries, others degenerated; a constantly changing vascular pattern was present.

For degenerating blood vessels, Sandison (1928a) reported a characteristic sequence. Circulation first ceased in the vessel; after a short time the lumen then narrowed and finally became obliterated, leaving a solid thread. Gradually this thread became thinner and broke, the stumps shortened, and the protoplasm and nuclei moved back into the mother capillary. Occasionally a temporary protuberance indicated where this had taken place, but in most cases the wall appeared smooth. This pattern for degenerating blood vessels is further substantiated by the reports of Clark and Clark (1939 and 1943).

Clark and Clark, in another series of articles (1932, 1933, 1937) presented their observations on the growth of lymphatic capillaries in rabbit ears, by means of the transparent chamber device. They reported that these capillaries sprouted as solid tips from preëxisting lymphatic endothelium, and gradually developed lumens by extension from those of the mother lymphatics. As the capillaries grew, they maintained pointed tips (Clark and Clark, 1937); when they had advanced

from a region of less dense connective tissue into a region of greater density, the tips became rounded and bulbous and growth ceased.

In 1932, Arey reported observing regeneration of connective tissue and epithelium. Wounds were made on either the fins or gills of fishes; and cysts, caused by glochidia, fresh water mussel larvae, were observed on amphibia. Epithelization occurred in two steps. First ameboid epithelial cells migrated into the wounded area and covered it, forming a scab. These cells did not proliferate, but the tissue from which the epithelial cells had come, did proliferate, thereby compensating for the cells lost by emigration; the same procedure was reported to occur for connective tissue. Gradually germinal cells grew underneath the scab, and the final epithelium was formed by these cells, which displaced the scab.

Stearns (1940) worked on rabbits and observed, by means of the transparent chamber, the formation of connective tissue fibrils in the rabbit's ear. She reported the formation to be already extensive in only three to four hours. After 48 hours, the mass was so dense that the fibroblasts were almost obscured. In twelve days a heavy layer had formed. As the tissue started to form, the fibroblasts increased in size, flattened, became striated, and joined each other by broad cytoplasmic connections (vesicular masses of cytoplasm,

which can be removed from the fibroblast proper) to form a true syncytium. Vacuoles were present in the cells but took no visible part in the fibril formation. As the fibrils formed, the cytoplasmic projections from the fibroblasts disappeared. The fibrils originated at random as extracellular material, due to fibroblast activity.

Dann, Glucksmann, and Tansley (1941) did work on regeneration of wounds caused by both mechanical injury and thermal burning. In the first four days following mechanical injury to the skin of rats-- either superficial injury to the panniculus carnosus, or a deep wound involving removal of a layer of skeletal muscle-- the contraction of the wound edge occurred, and the beginning formation of the epidermis in a thick ring just inside the wound edge. Within 9 days, epithelial regeneration was completed, by the formation of an abnormally thick sheet of tissue which had reduced in thickness by the fourteenth day. The return to normal thickness did not occur rapidly, for on the fiftieth day, the difference in thickness between the regenerated tissue and the normal tissue was still discernible. In the case of a deep wound, the observers reported the appearance of new muscle fibers by the fourteenth day.

In healing of tissues injured by thermal and chemical means, with intent to burn, regeneration of epithelial and

connective tissues, blood vessels, and muscle fibers have been observed. (This does not mean, of course, that regeneration of nerves and lymphatic vessels as well does not occur following a burn, but simply that no attempt has yet been made to observe regeneration in these other cases.) Work has been done in this field by Dann, Glucksmann, and Tansley (1941), Hughes and Dann (1941), Glenn (1944), Patey and Scarff (1944), Converse and Robb-Smith (1944), Bishop (1945), Harkins (1945), and Strassmann (1933).

Dann, Glucksmann, and Tansley (1941), by methods described elsewhere (viz., "Destruction"), found that healing is slower in burns than in wounds made by mechanical means. Contraction does not begin until the tenth day after burns, and epithelization is not complete until the twenty-seventh day; for not even a scab forms until the tissue originally destroyed by the heat has fallen out, an occurrence which precedes contraction of the wound. Glenn (1944) corroborated the retardation of healing by the presence of dead tissue. Dann and his co-workers explained that enlargement occurred almost at once after a burn, and slight additional expansion followed upon the dropping out of the necrotic central material.

In the time preceding the displacement of this plug, "active mitosis" of the epithelium has occurred and resulted in a thickened ring around the necrotic center. Following the

expulsion of the plug (caused by the infiltration of leucocytes and the pressure of normal tissue underneath), healing proceeded rather rapidly; epithelium moved over the surface and collagenous fibers filled in the cavity. The rapidity of this process was directly proportional to the depth of the wound. Even by the sixtieth day, however, no elastic fibers had regenerated. Those blood vessels near the burned area were blocked by clots, which gradually disappeared, and the reëstablishment of circulation followed. Vessels within the central area were destroyed by the initial burning, and collateral circulation was developed around the necrotic center by capillary sprouts. The muscle fibers in the central area, like all other tissues there, were killed outright; other muscle fibers near the central area were damaged by conducted heat, and degenerated during the first twenty-four hours. Regeneration of the muscle fibers occurred around the periphery of the central plug, prior to its expulsion. Following the loss of the plug on the tenth day, mitosis of the myocytes occurred on the sixteenth day; and regenerated fibers were found as early as the twenty-first day. In short, after a burn, epithelial tissue began to repair almost at once, connective tissue in approximately five days, and a few new fibers of muscle could be located after seven days in muscles of the skin and in ten days for skeletal muscles.

Hughes and Dann, in 1941 (see "Destruction" for a description of method), agreed with Dann, Glucksmann, and Tansley (1941). They reported dilatation of the capillaries following a burn and the union of partially destroyed vessels around the necrotic center through capillary sprouts which become connecting loops. The vessels injured but not destroyed are filled with clotted blood. Both fibrous tissue and blood vessels start to regenerate in the wound on the tenth day, following the expulsion of the necrotic plug. (There may be some regeneration of the vessels in the deep area before the plug is expelled.) Regeneration begins, in the case of the blood vessels, in the form of a thick vascular network containing pointed capillary sprouts and growing from the cut edges of the panniculus into the wound proper. The sprouts regenerate in a manner like that reported by Clark and Clark. In twelve to sixteen days full vascularization has taken place. Epithelization and fiber formation have occurred superficially by this time, but the vascularization and fiber formation in the deeper layer does not occur until later, at which time the two processes proceed concurrently. These areas of vascular repair contain material composed of parallel layers of fibrocytes, some of which form the endothelium of the newly forming capillaries. As the healing progresses, tissues in the regenerating area appear more and more normal.

It is apparent from the data of Hughes and Dann (1941) either that no scab formed like that described by Arey (1932) in the case of burns, or perhaps that the animals were not observed until the scab was completely gone. The latter case seems more probable, both from common everyday experience with skin burns, and from the evidence presented by Strassmann in 1933 (see "Destruction" for description of method) where a scab was observed to form at first, and leucocytes were seen to migrate into the scab, which was finally sloughed off in approximately eight days, leaving a non-characteristic scar.

In his work on regeneration of skin in man, Bishop (1945) used the dorsal surface of his own forearm. The skin was removed either by cutting or by chemical burning (a saturated solution of barium or sodium sulphide on gauze was placed over the area to be injured). Speed of regeneration was inversely proportional to the depth of injury, and proceeded in the following manner. If the wound were so shallow that papillary tissue remained, regeneration occurred smoothly over the surface; if the wound were such that the papillary layer had been removed, mounds of granulation tissue developed in the region of the hair follicles, even in spite of the destruction of the epithelial tissue at the base of the hair follicles. Epithelial cells then migrated into the wound and imbedded themselves in the granulation tissue, eventually

covering the wound. These migratory epithelial cells may come either from the undamaged epithelium at the margin of the wound, or from remaining epithelium at the base of the hair follicles. In either case, the division of cells occurs before, and not after, the cells have migrated to the granulation tissue. This bears out the work of both Patey and Scarff (1944) and Converse and Robb-Smith (1944), for they reported that some epithelium regeneration was derived from hair follicles.

A scab may be formed, as Bishop (1945) further reported, before the final epithelization occurs. This happens if migration of epithelial cells takes place before healthy granulation tissue has formed. This grows as a "free lip" without attachment to the underlying tissue.

Regenerating epithelium which will result in scar tissue, and that which will result in normal tissue, both have at first identical granulation tissue. It is the actual deposition of an excessive amount of fibrous material by dense bundles of horizontal collagenous fibers developing in the granulation tissue, and the gradual obliteration of blood vessels, that gives a scar its characteristic appearance. This does not occur until after the surface epithelium has completely covered the wound.

The success, and the pattern, of healing, then, depend

more on the extent and depth of injury than on the means of injury, as was indeed reported by Bishop (1945). The work to be discussed in this thesis (in "Observations", q.v.) will further substantiate the conclusion here stated. Dann, Glucksmann, and Tansley (1941) demonstrated that burns are more susceptible to infection than other types of wounds, and this is probably the main reason for slowness of healing in burns.

OBSERVATIONS

METHOD

Anesthesia: Medium sized frogs were anesthetized by injecting into the dorsal lymph sac either, approximately, 0.7 cc. of a 20% aqueous solution of urethane or 0.02 cc. of a 10% alcohol solution of veterinary nembutal. To render the animal unconscious, urethane required 10 to 20 minutes; animal nembutal 40 to 60 minutes. The former kept the frog unconscious for 20 to 30 minutes and the latter for 1 to 3 hours.

Discussion: Both drugs have their advantages and disadvantages. Urethane does not depress the circulation as much as nembutal, nor does a supposedly anesthetic dose so frequently prove to be a shocking or lethal dose. On the other hand, nembutal will keep the animal anesthetized for longer periods, and the recovery from the effects of the drug is much more gradual. Both drugs were tried for four month periods, and urethane was found to be more satisfactory.

In using either of the drugs, the following factors had to be considered: (1) depending on the size of the animal, a dose larger or smaller than 0.7 cc. of urethane or 0.02 cc. of animal nembutal was required; (2) female frogs filled with eggs required from one and one-half to two times

larger doses than males of the same size; (3) frogs, to all appearances identical, might have different sensitivities to the drugs; (4) there was a slight daily variation in an individual frogs sensitivity to the anesthetizing dose; (5) if the normal anesthetizing dose was split and administered at short intervals, it was no longer sufficient to anesthetize the frog.

Preparation: The retrolingual membrane of Rana pipiens was chosen for experiment, because of its easy accessibility and arrangement; for the muscle fibers, blood vessels, and nerves are contained in a thin transparent membrane between protecting epithelial layers (Lutz, 1942). Following the method of Pratt and Reid (1930), the epithelium, the muscles, and the connective tissue were removed from the outer surface of the tongue of an anesthetized frog, revealing the retrolingual membrane, which was then transilluminated by the Fulton and Lutz method (1940). The animal was placed, with either ventral or dorsal surface up, in a petri dish to which a glass block had been cemented, and around which paraffin had been laid. The tongue was gently stretched over the glass block and held flat and firm by small pins placed through it and into the paraffin surrounding the glass block. The entire dish was filled with frog Ringer's solution until the membrane

was submerged enough to present a smooth surface. If the day were warm and circulation ceased, several frozen cubes of the Ringer's solution were added. The Petri dish containing the frog was then attached to the microscope stage either by means of small springs and a semicircular wooden block, or by a round flat wooden container held to the microscope stage by clips (Fulton, 1941). Nothing further was done to the control membranes, except in removing clots, when possible, and in gently separating the wound edges when they adhered. The membranes were observed until healing obscured them or death of the frog interfered. If ligation of the large blood vessels was necessary to clear the membrane, the frog in question was not used.

Burning: A small spot, 100 to 200 microns in diameter, was burned whenever possible on a portion of the membrane in which the circulation was active. The burning was done in either of two ways: by means of a brief faradic current from an inductorium (with a key and one dry cell in the primary circuit, and in the secondary circuit a small flat metal piece placed under the tongue, and a silver and glass microelectrode made by the Fulton method, 1941); or by a wooden-handled dissecting needle raised to red heat over an alcohol lamp.

Both the control and the burned frogs were observed

daily, whenever possible, except for weekends. Between observations, unless otherwise stated, the frogs were kept in approximately $\frac{1}{4}$ to $\frac{1}{2}$ inch of water in individual wire-covered glass containers, which were kept in the lower half of an electric refrigerator at a temperature of 12 degrees Centigrade. The frogs were kept in the refrigerator in order to slow their metabolic processes sufficiently so that daily observations would be adequate to record their organic changes.

Discussion: Neither of the burning methods was completely satisfactory. In the case of the electrode and inductorium, it was difficult to avoid giving the frog a severe electric shock, due to spread of current through its body, when the inductorium was adjusted to provide sufficient secondary current to burn the membrane. The disadvantages of the dissecting needle and alcohol lamp were too rapid cooling, less accuracy in manipulation, and deposition of carbon granules picked up from the flame of the alcohol lamp. These latter drawbacks, however, were nevertheless preferable to shocking the frog, and all but three frogs were burned in this manner.

PRELIMINARY OBSERVATIONS

Before the effect of burning could be accurately judged, it was felt necessary to observe membranes prepared completely except for burning, which would thereby serve as controls. A

total of twenty-four control frogs were observed and their records kept (see Table I) during the period from January 31 to June 3. Of these, eleven lived an average of ten and a half days; two frogs lived twenty and twenty-seven days, respectively; five lived an average of one to two days; and the remaining six were discarded. Of the frogs that died, four had pronounced cases of "red leg". The deaths of the remaining fourteen could not be ascribed to so definite a cause.

The membranes of many of these control frogs varied in the daily appearance of the blood vessels, but only very infrequently in the appearance of the other tissues. Once a fractured muscle fiber was present, and occasionally, if the frog lived close to ten days, the membrane would become cloudy owing to extravasated erythrocytes, leucocytes, and active ciliated epithelial cells. With regard to the blood vessels, nine of the twenty-four frogs had capillary sprouts; of these, six had degenerating sprouts and three, elongating sprouts. (Any incomplete vessel was referred to as a sprout.) Eleven of the frogs had thread-like capillaries; and of these, four had a great number of the thread-like capillaries, and four others had both thread-like capillaries and capillary sprouts. The patterns of circulation observed, particularly on Frog I (see maps of Frog I) indicated that vessels in the same areas of the membrane tended to remain active from day to day, and that vessels in still other areas tended to remain inactive.

Inflammation was present in some membranes, in the

marked swelling of the tongue; but in others only slight dilation in the lateral veins and arteries occurred, and this may have been due to mechanical stimulation (which in the frog causes dilatation), when the tongue was pulled out and pinned. Healing occurred in either of two ways. In the first type, particularly noticeable in frogs one and seven, the cut edge of the outer surface of the tongue would encroach on the membrane and gradually cut down the field (see maps of frog I). The other type of healing occurred in frogs left over the weekend, during which time the tongue remained in the frog's mouth relatively undisturbed, and the cut edges of the outer surface were in close proximity. By the following Monday, the edges would often have adhered, and would have to be pulled gently apart. The new tissue joining the edges on these occasions was colorless, translucent, and slightly glassy. It seemed to be loose connective tissue not in contact with the membrane, and upon its removal, the area of the cleared membrane appeared as large as on the previous Friday. No frog lived until complete healing of the wound had taken place.

Discussion: It would seem, then, from these observations, that the control membranes under examination normally have a fairly large proportion of capillary sprouts and threadlike capillaries. But Professor Lutz and Dr. Fulton have pointed out that in the spring and early summer capillary sprouts are much more frequent than at other times of year, and that this is the season when "red leg" is most prevalent.

Clark and Clark (1939) report that prolonged irritation or chronic inflammation, such as would be caused by "red leg", seem to increase the vascularity of a tissue, by causing capillary sprouts to appear. On the other hand, Professor Young has suggested that there may be some correlation between sex hormones and the occurrence of capillaries at this season. It is uncertain whether either of these suggested correlations is valid, but it is true that membranes observed at other times of year have shown capillary sprouts only infrequently.

Thread-like capillaries were observed at all seasons of the year, and were found to be particularly numerous in frogs which had been given too large a dose of urethane or nembutal. In the membranes of frogs 20, 21, 22, 24, and 25 this was probably the case, for frog 24 lived 3 days and the others only one day each. Since these frogs were previously lively and to all appearances in good health, the anesthetizing dose must have been too large, causing shock. The blood vessels in the membranes of these frogs and other shocked frogs appeared narrow, stretched, without circulation, and exhibited a characteristic pattern resembling a line connecting the points of two "V's" (see Figure I). The vessel forming the line between the "V's" occasionally became increasingly slender and attenuated and finally broke into segments and disappeared. No frog lived long enough for it to be seen whether

the two "V"-shaped vessels lost their pointed appearance, nor whether actual retraction occurred, as Clark (1939) observed in the case of a rabbit ear.

Summary of Preparatory Observations:

1. Control membranes changed most frequently in the appearances of the blood vessels, with both regeneration and degeneration occurring, and also, but infrequently, in the appearance of other tissues.

2. Inflammation occurred in control membranes, being indicated by the typical symptoms.

3. Healing of the wound made by the original dissection occurred in one of two ways: by the gradual encroachment over the cleared portion of the membrane of the tissues comprising the outer surface of the tongue, or by the growth of tissue between the closely juxtaposed wound edges of the undisturbed tongue, leaving the membrane free.

4. No frog lived long enough to permit complete healing of the wound.

5. With a shocking dose of urethane or nembutal, thread-like capillaries appeared in profusion in the membrane, and there was occasional degeneration of the capillary forming the connecting link between "V"-shaped vessels at either end.

THREAD-LIKE CAPILLARIES

IN

CONTROL MEMBRANES

Fig. I: Illustrates a thread-like capillary in a frog anesthetized with 0.7 cc. of Urethane. The red cells in the capillary did not move during the observation. The circulation indicated by arrows was very active. This was a new preparation and was observed on the first day.

Fig. II: Illustrates a thread-like capillary in a shocked frog, with the typical "V-line" appearance clearly indicated.

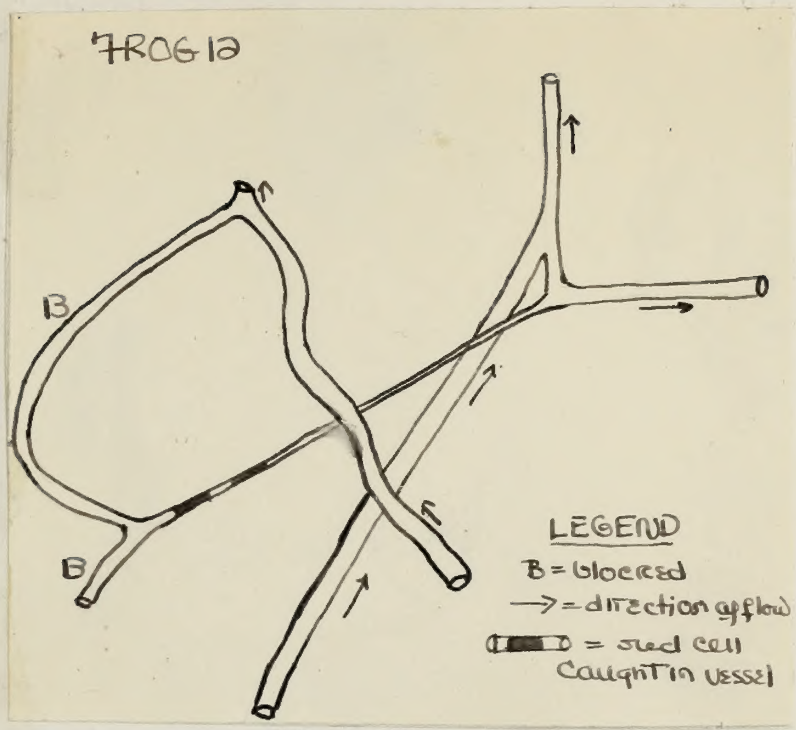


FIGURE I

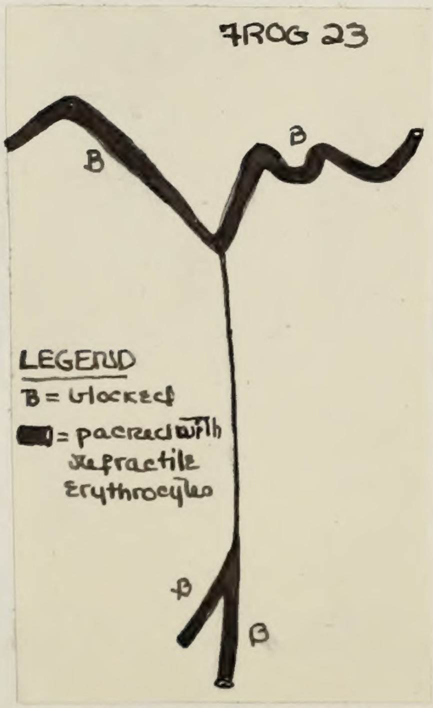


FIGURE II

CAPILLARY SPROUTS

IN

CONTROL MEMBRANES

Fig. IV: Illustrates a blunter tip filled with erythrocytes, off of a partially blocked vessel.

Fig. V: Illustrates a tip like that of Fig. IV, but a sprout off of a vessel that was at first blocked and finally carrying.

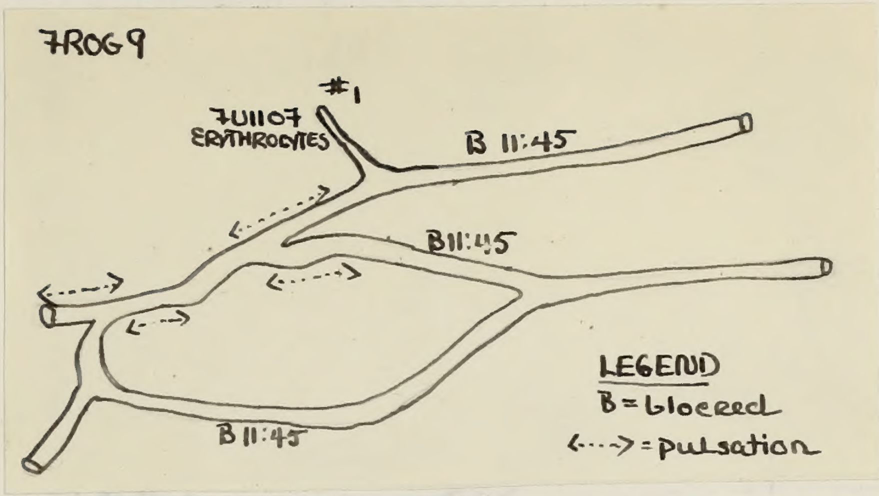


FIGURE IV

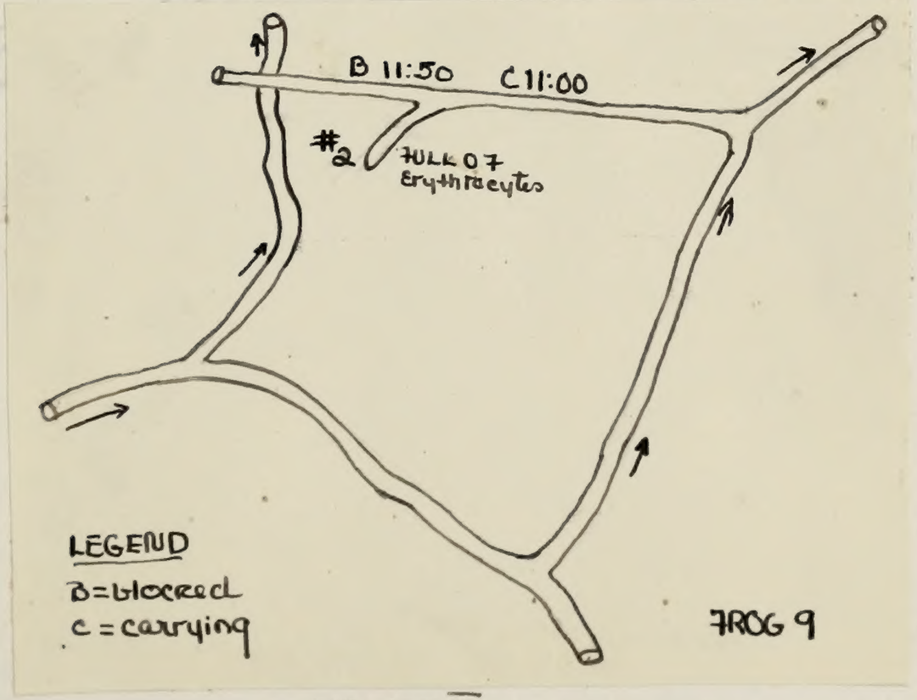


FIGURE V

Figs. VI and VII: Illustrate the joining of two sprouts with the great mass of extravasated erythrocytes involved in the growth.

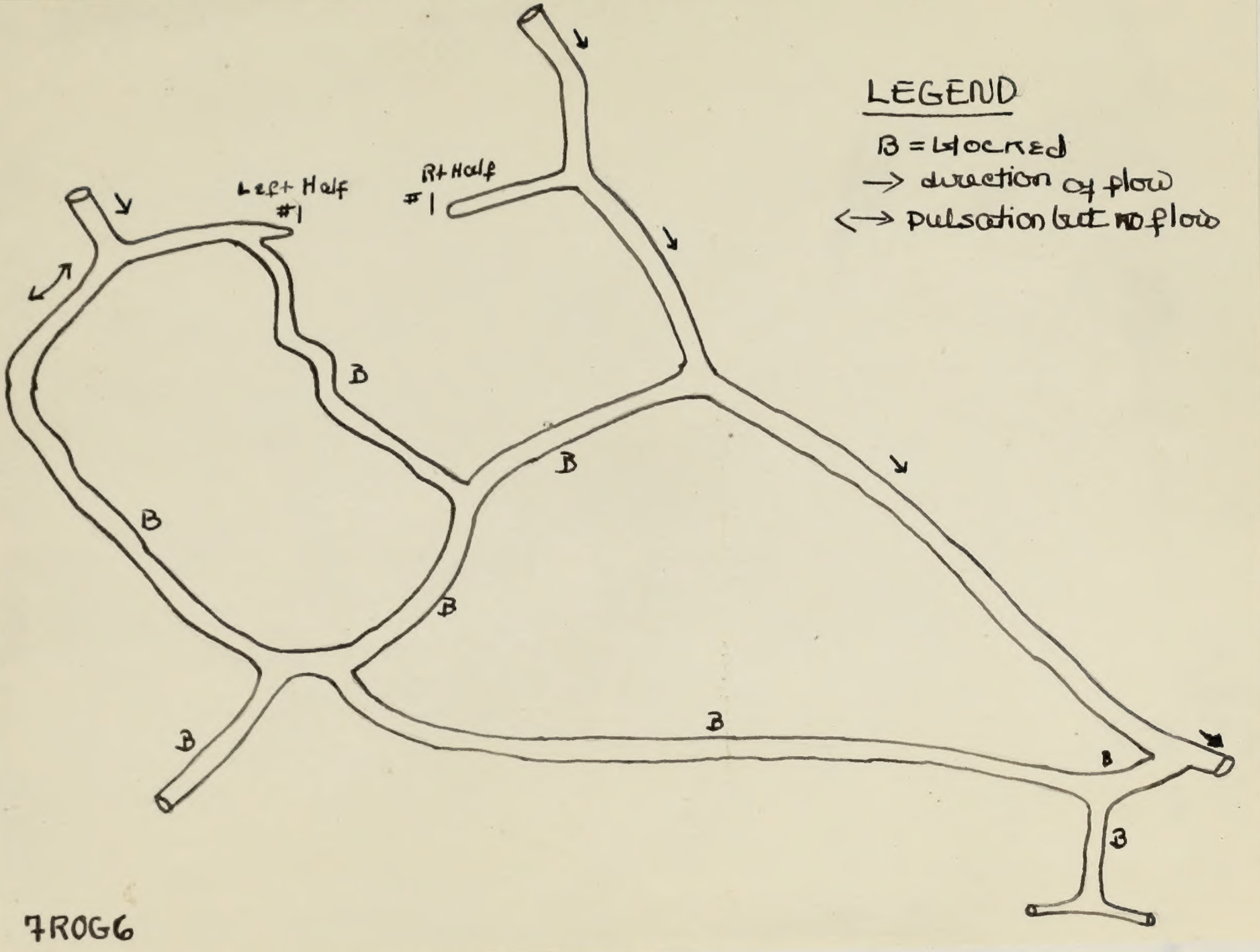


FIGURE VI

FROG 6 (cont. next page)

FROG 6   Eight days later

Showing final joining between sprouts #1 and #2

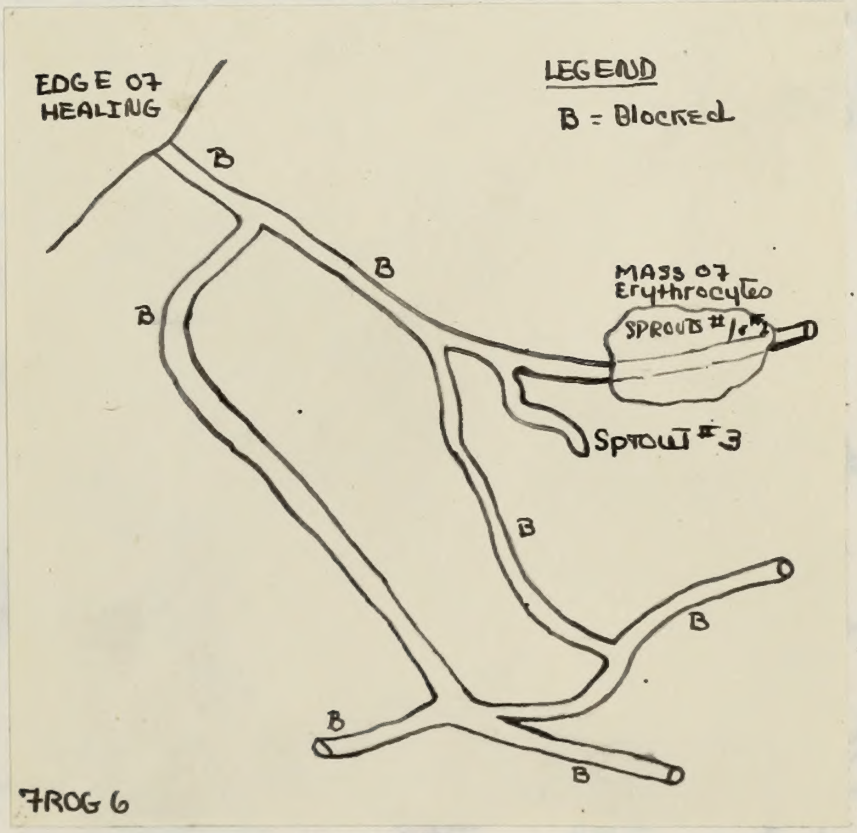
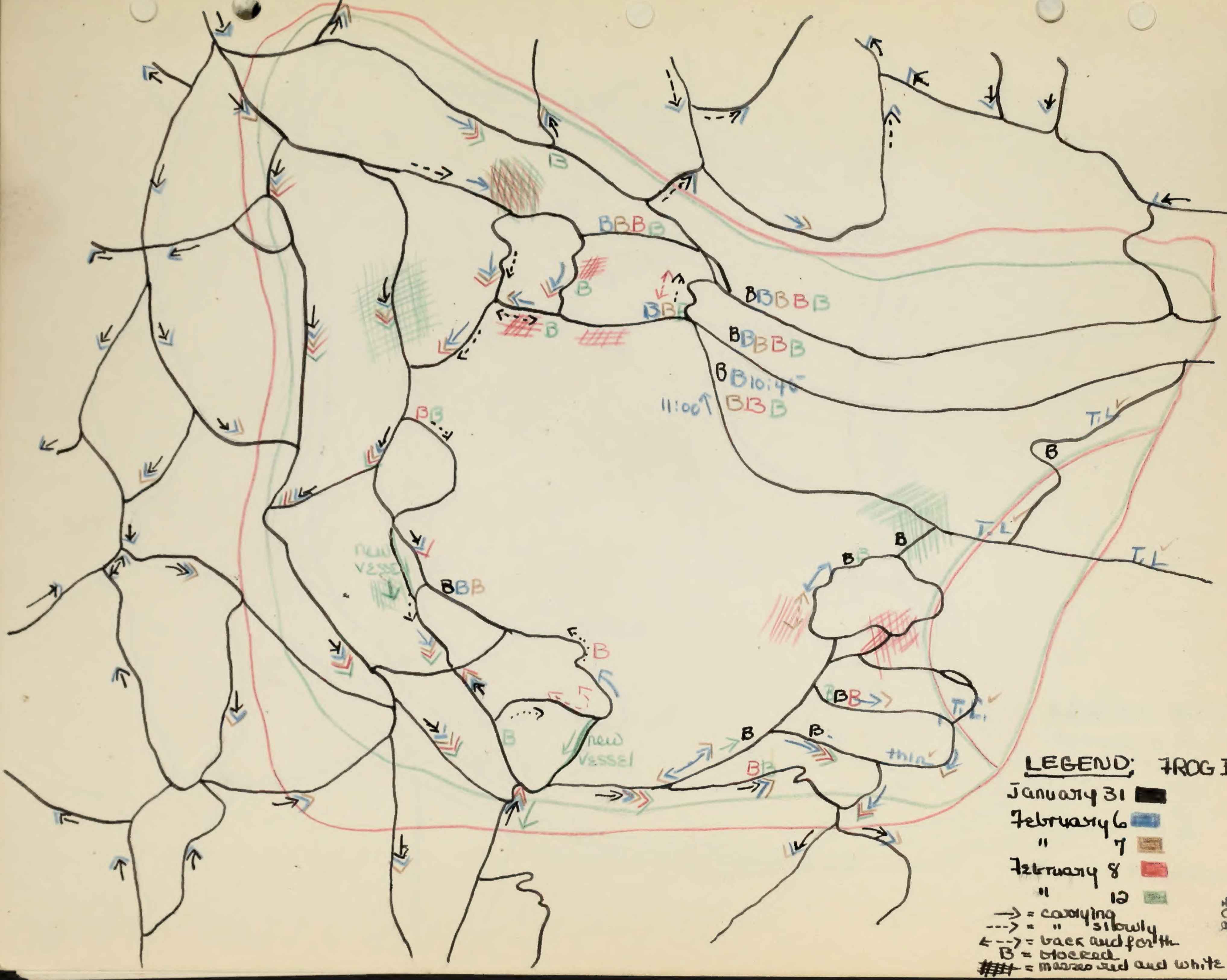


FIGURE VII

This map and the one on the following page serve as a good illustration of the type of healing that is due to encroachment of tissue from the outer edge of the membrane. This frog lived 20 days, and maps of January 31, February 6, 7, 8, 12, 13, 14, 15, 18, and 19 are shown in these two figures. Two new vessels were noticed on February 12 but they appeared complete and were carrying blood. Since no sprouts were observed, the vessels must have started on the 9th. Thread-like capillaries were observed, but no degeneration or growth of these were seen. The slowing down of the healing process due to infection is nicely illustrated, for from February 13 to 19 only slight healing took place. The frog was dead on February 20.

MAP I



LEGEND; FROG I
January 31 ⬛
February 6 🟦
" 7 🟫
February 8 🟥
" 12 🟩
→ = carrying
---> = " slowly
←--> = back and forth
B = blocked
= massed red and white cells

MAP II

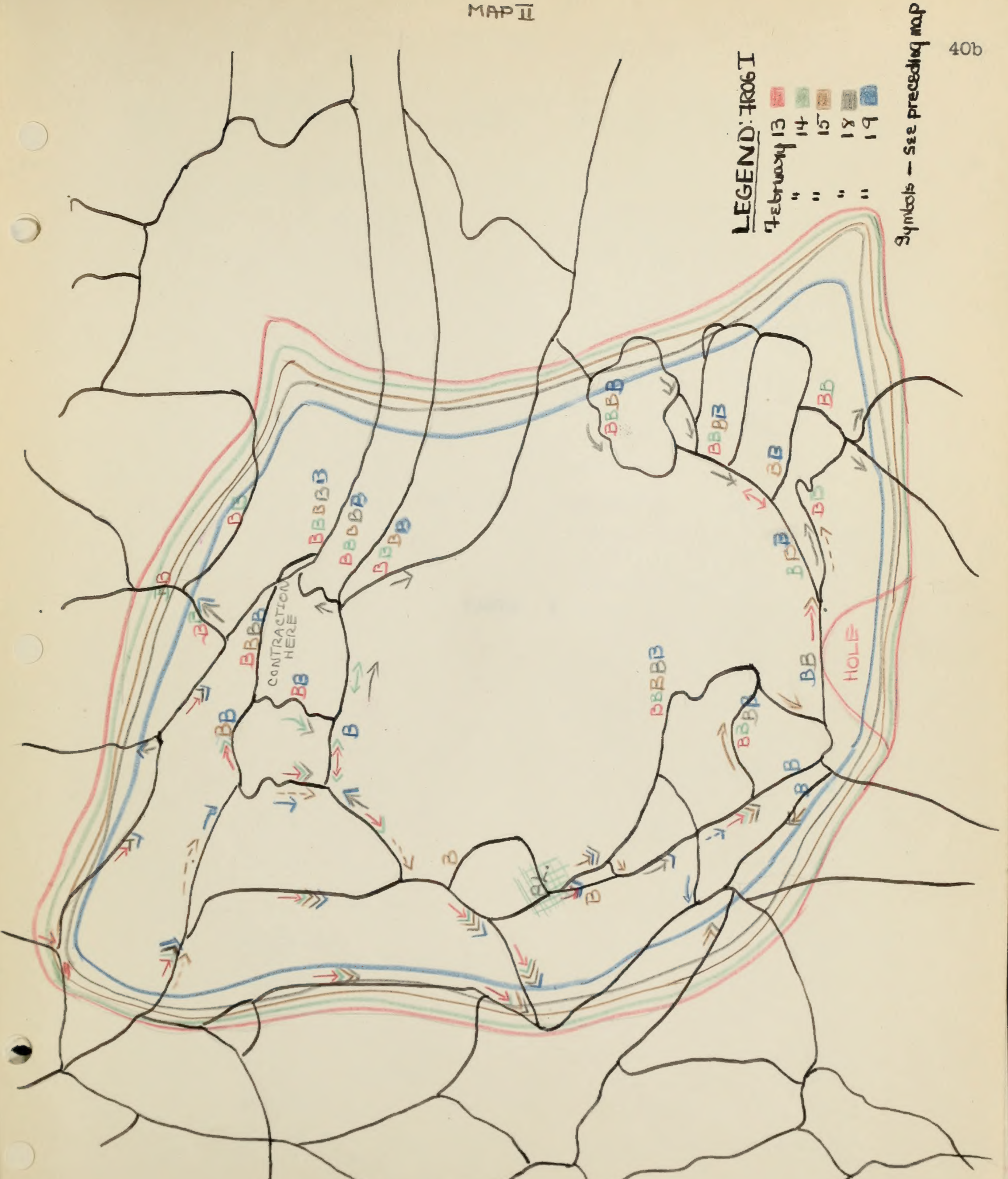

LEGEND: FROG I
February 13 🟥
" 14 🟩
" 15 🟫
" 18 ⬛
" 19 🟦
Symbols — See preceding map
CONTRACTION HERE
HOLE
BBBBB
BBBBB
BBBB
BBBB
BBBB
BBBBB
BBB
BB
BB
BB
B
B
B

TABLE I

TABLE I

Frog No.	Capillary Sprouts			Thread-like Capillaries
	Number	Degenerate	Grow	
1	0			2
2				
3	1	X		0
4				
5	1			0
6	3	X 1-11 da*	X 12 da*	0
7	2	X		1
8	1		X	0
9	2	X 3-9 da*	X 1-2 da*	0
10				0
11				0
12	1			2
13	1	X		1
14				0
15				
16				0
17	1	X		1
18				0
19				0
20				Numerous
21				Numerous
22				
23				
24				Numerous
25				Numerous

* Days after original dissection, inclusive.

TABLE I

Frog No.	No. Days Lived	Disposal	Anesthesia	Remarks
1	20	Died	Urethane	
2		Discard	"	Ligation necessary. Frog therefore discarded.
3	7	Died	"	Red leg
4		Discard	"	Blood clot. Frog therefore discarded.
5	5	Lost	"	Frog lost: disappeared from container.
6	14	Died	"	Joining of sprouts #1, #2
7	26	Died	"	Thread-like cap.- dilated Partial healing
8	7	Given away	"	Red leg
9	9	Died	"	Red leg
10	8	Given away	"	Good circulation throughout. Partial healing
11	1	Died	"	Partial healing
12	13	Died	"	Closing of membrane by 2nd healing method
13	10	Died	"	Red leg (10th day)
14	16	Died	"	Swollen lymph sacs
15		Died	"	Control: just inj.
16	3	Died	"	Extravasated erythrocytes bleeding
17	12	Died	"	Connective tissue fiber bet. thread-like caps. lost
18	10	Died	"	
19	5	Given away	"	
20	1	Died	"	Lethal dose, urethane
21	1	Died	"	Lethal dose, urethane
22		Lost	"	Disappeared
23	1	Discard	"	Died. Bled profusely: therefore discarded.
24	1	Died	"	Fractured muscle fiber
25	1	Died	"	Lethal dose, urethane

OBSERVATIONS

Immediately following the burning of the membrane, the area affected appeared cloudy and spotty; portions of the vessels were destroyed; circulation ceased in the immediate vicinity; some extravasated erythrocytes appeared, and, lastly, some white cells and ciliated epithelial cells. In a burn of sufficient intensity, a triple area effect also appeared, becoming quite obvious within a few minutes after the burning. This effect consisted of (1) a granular and distorted center (the portion touched by the cauterizing needle), surrounded by (2) an opaque, shelf-like ring, and this, in turn, by (3) the rest of the membrane. The third area did not have a definite limit, but was that portion of the membrane on the outer border of the opaque ring, which still contained distorted blood vessels and cells. This third area became progressively more normal at greater distances from the opaque ring. (See Figure, page 57.)

In the granular and distorted central portion of the burned area, all vessels and muscle fibers were completely destroyed. Occasionally all tissues in the area were destroyed, leaving a hole. This happened in one of two ways: the tissues might stick to the cauterizing needle and be pulled out when the needle was removed, or on days succeeding the

burning the tissues might disintegrate.

In the region of the shelf-like, opaque ring, vessel walls could be seen. In some cases they would extend to the central area before disappearing, but when this happened the lumina of the vessels would gradually become obliterated as they approached the central area, leaving a single thread, which was only intermittently visible. The lumina of these vessels contained fragmented erythrocytes; and muscle fibers in this area were no longer in one piece, but divided into segments, coagulated, and, at the ends of the coagulated portions, glassy.

In the portion of the membrane just bordering the opaque area, vessels and muscle fibers might appear slightly pulled out of shape after burning, compared with their previous appearance. Since the border of the opaque area varied slightly from day to day, it was difficult to locate in all cases the previously observed segment of the vessel, and therefore difficult to determine whether the damaged segment had repaired, or whether the observer was merely looking at a different segment of the vessel.

On the second day the carbon granules were either diminished or absent; white cells and epithelial cells were found in the vicinity of the burn with ingested carbon particles; ciliary action was observed; circulation was still ab-

sent, the damaged vessels being not yet patent, although there was some pulsation of blood at the entrances to the damaged vessels. More extravasated erythrocytes had appeared. If a hole had been made the first day, the edge of the hole was ragged and slightly smaller. The triple area effect was still present, but in cases where there was no hole the central portion was no longer so clearly defined. The outer edge of the opaque area appeared to contain many epithelial cells with actively beating cilia, white cells, extravasated erythrocytes, and other tissue cells so badly damaged as to be unidentifiable.

By the third day, if a hole had been present, connective tissue fibers were found across it, and in one frog a muscle fiber had so formed. Circulation was still absent in and near the region, but some of the vessels were beginning to show degeneration (shortening and becoming thread-like) or regeneration. In regeneration of blood vessels, the tip of the vessel was not found to be losing its lumen, the walls behind the tip remained definite, and extravasated erythrocytes collected at the tip. The triple area effect was still present, but there had usually been a slight shrinkage of the opaque area, with a shelf-like appearance still marked, however, and very active ciliated epithelial cells along the edge of the mass.

By the fourth day, the triple area had largely disappeared, with marked degeneration or regeneration of the vessels in the opaque area, the latter being particularly evident in repair of vessels adjacent to the opaque area, some of which were now carrying blood. The vessels actually within the opaque and central areas were still slightly granular in the portions still existing, or appeared as single threads. The border of the opaque area might still be partially visible, and if so there were usually numerous active ciliated epithelial cells in the border.

By the fifth day, circulation was usually more active in the outlying vessels, but there had been generally very little change in the other tissues.

Only two frogs lived more than five days (for complete protocols see pages 51-55, 59-60) and one of these (see Figs. IX to XI) had a rather bad infection on the ninth day. The membrane of this frog was cloudy, owing to red cells, leucocytes, and epithelial cells, and had yellowish mucus covering it. The arteries and veins lateral to the membrane were engorged with blood. All of the four arteries supplying the membrane, however, were carrying blood. The ring of epithelial cells outlining the opaque area had disappeared, but there were scattered epithelial cells with active cilia still above the normal surface of the membrane. The damaged blood vessels

still appeared the same as they had on the fifth day. On the tenth day, this frog was dead, still without complete healing of the membrane.

The other frog died on the sixth day (see Figures XIIa, b, c). There was no evidence of infection, and the burned area on the membrane was healing much more rapidly. On the fifth day, reëstablishment of the circulation had taken place in one of the damaged vessels, and another to all appearances was repaired, but still not carrying blood. The hole (corresponding to the center area) was no longer present, but the former outline could be discerned by a slight thickening of the membrane. Disintegration of some of the damaged vessels was continuing, and extravasated erythrocytes were present around portions of both disintegrating and growing vessels.

Summary of Observations: Tissue destruction, then, was observed initially following the burn, with all the tissue cells in the region having a granular, or glassy, appearance and a distorted outline, the area consequently appearing cloudy and spotty; with a severe burn, the three area effect is observed, vessels were destroyed, and circulation ceased. Increased capillary permeability resulted, and leucocytes and the erythrocytes appeared on the membrane in the burned area.

This, plus the swelling, plugging of the vessels, and the migration of ciliated epithelial cells in and near the area, provided the typical picture of inflammation. If infection occurred, inflammation was intensified and healing slowed. Regeneration occurred in blood vessels, connective tissues, and epithelial tissues, and was once observed in a muscle fiber. In the case of the blood vessels, both regeneration and degeneration were observed. In either case, extravasated erythrocytes were present around and at the tips of the vessels; these disappeared, however, from the portions of the degenerating vessels which had become thread-like, and these thread-like portions would gradually break into smaller portions and finally disappear. Since no frog lived long enough for complete healing to occur, it was impossible to judge accurately how normal in appearance the regenerated epithelial and connective tissues were.

DISCUSSION

The observations on the burned membrane reveal the characteristic pattern of destruction, inflammation, and regeneration found in other burns. When infection was present, it produced the usual slowing of the healing process. Epithelial and connective tissues, muscle fibers, and blood vessels repaired following the burn. The main difference between the healing of epithelial tissues as reported by Bishop (1945), Patey and Scarff (1944), Converse and Robb-Smith (1944), and as observed in the membranes in this research is that there were in this case no hair follicles present, and regeneration therefore took place only by the migration of the ciliated epithelial cells into the region.

Since little research on burns has been done by daily, or more frequent, observations of living tissues, this is a relatively unexplored method. Problems, such as knowledge of step-by-step regeneration of all five types of tissues, and a more detailed study of the effect of infection, might be investigated.

As for the original research given in this thesis, the work needs to be repeated on many more frogs, until at least thirty more cases like frogs six and seven are produced. This would greatly increase the validity of the reports. Observa-

tions two or three times daily should also be made, not only on frogs kept at a temperature of 12° Centigrade, but also on frogs at approximately 20° Centigrade. The work might be further extended by using the same method on the hamster cheek pouch as was used on the retrolingual membrane of the frog. This would serve as a check on differences in the effect of burning and regenerative pattern between amphibia and mammals. This investigation would present a more complete step by step account of destruction, inflammation, and regeneration in living tissues.

BURNING

Typical Protocols
And
Figures

FROG VI

November 4, 1946

10:15 .02 cc. nembutal Dissection made

10:45 .01 cc. " Before and after drawings (Fig. IX, and Figs. X, XI) Burned.

Before: Circulation is almost entirely absent.

1. In the center of membrane (including burned area), there is none.
2. Several vessels are carrying along the edge of the membrane.
3. Vessels lateral to membrane are carrying.

Area chosen is clear.

After: Circulation is the same.

Area burned:

1. Clear area has developed at the place where the point of the needle touched.
2. Vessels in this (the clear area) and the surrounding area were destroyed.
3. Burned area may be divided into three parts: the first is a clear central area; the second is an area lying like a cloudy halo around the clear area and ending in a shelf-like effect. Blood

vessels were destroyed in this area. In the third area, the outermost one, the vessels were not destroyed. Muscle fibers just outside the second area appear slightly distorted, but moving farther away from the second area, one finds the effects of the burn to be less and less noticeable.

November 5, 1946

10:10 .02 cc. nembutal Frog groggy, at first thought dead-- pinned tongue out, but he wriggled, so anesthesia was given (he had evidently warmed up).

Circulation is present above and below the burned area due to two active arterioles, but is absent in the burned area itself. The heart action is strong.

Area: The 3 area effect is still present: clear central area, cloudy ring, and approximately normal area on outer border of ring. The central area is not quite as marked today. There is, however, definite destruction of blood vessels. The cloudiness of the left portion of area two has lessened and

the entire area has changed in outline (see Fig. X). Muscle fibers are seen running through area two. Blood vessels in this area are visible, portions are destroyed; those vessels that are still existent are filled with fragmented red cells.

November 6, 1946

The frog appears dead, for there is no visible heart action. There is, however, even, but slow, circulation in arteries of the tongue. Connective tissue fibers are starting to grow across the dissection wound and the edges had to be pulled apart and the membrane cleared. There is ciliary activity in region of cell band, and in the band proper, indicating that living epithelial cells have partially formed the band.

November 7, 1946

The frog looks dead, but there is slow movement in large lateral arteries. Connective tissue fibers growing over dissection. The pattern is now like Fig. XI. The ring of epithelial cells is almost gone except in lower section.

By 11:30, the heart action was rather strong but there was still no eye or leg reflexes nor any circulation through the

membrane.

November 8, 1946

The heart action is still visible and a leg reflex occurs, but no eye reflex; therefore the anesthesia was omitted. The frog's body was bloated. The membrane sides are again held by connective tissue fibers. Vessel "I" still is not patent, and appears as it did yesterday. The ring of epithelial cells in lower left is still visible, and is slightly more marked than yesterday. The circulation in the membrane is much better. 3 supplying arterioles are carrying.

November 12, 1946

The body is badly swollen, and the frog is so groggy that an anesthetic is not needed. The tongue has grown together slightly at the ends and is largely covered by yellowish-looking mucus. This was easily removed. All four incoming arterioles are carrying. The membrane is cloudy; red cells, epithelial cells, and leucocytes are present, so it seems that the yellowish-looking mucus is an indication of infection. Both arteries and veins along the sides are swollen. The ring of epithelial cells has disappeared. Ciliary action is still marked, both in the masses of epithelial cells above the surface and in those on the surface of the membrane.

November 13, 1946

Frog VI is dead; there is neither circulation in lateral arterioles and veins of tongue nor visible heart action, and there are no reflexes. The membrane is covered with numerous red cells formed into a type of surface covering, by aid, it seems, of connective tissue fibers, for the covering does not look like a clot, and was removed in one piece with forceps. There was twitching of muscle fibers in one region and a partial draining of the venule over it. When the twitching stopped, the draining slowed down, and in 30 seconds had stopped.

In the burned area the blackened portion of vessel "I" (see Figure XI) is shorter. The clearer area in center where the point of the burning instrument touched can still be vaguely discerned, and the band of cells on the lower left (Fig. XI) still have active cilia.

Figure IX. The before-burning pattern of a membrane with two thread-like capillaries already present, and completely blocked vessels surrounding the capillaries. For a more complete description of this and the following figure, see the protocol.

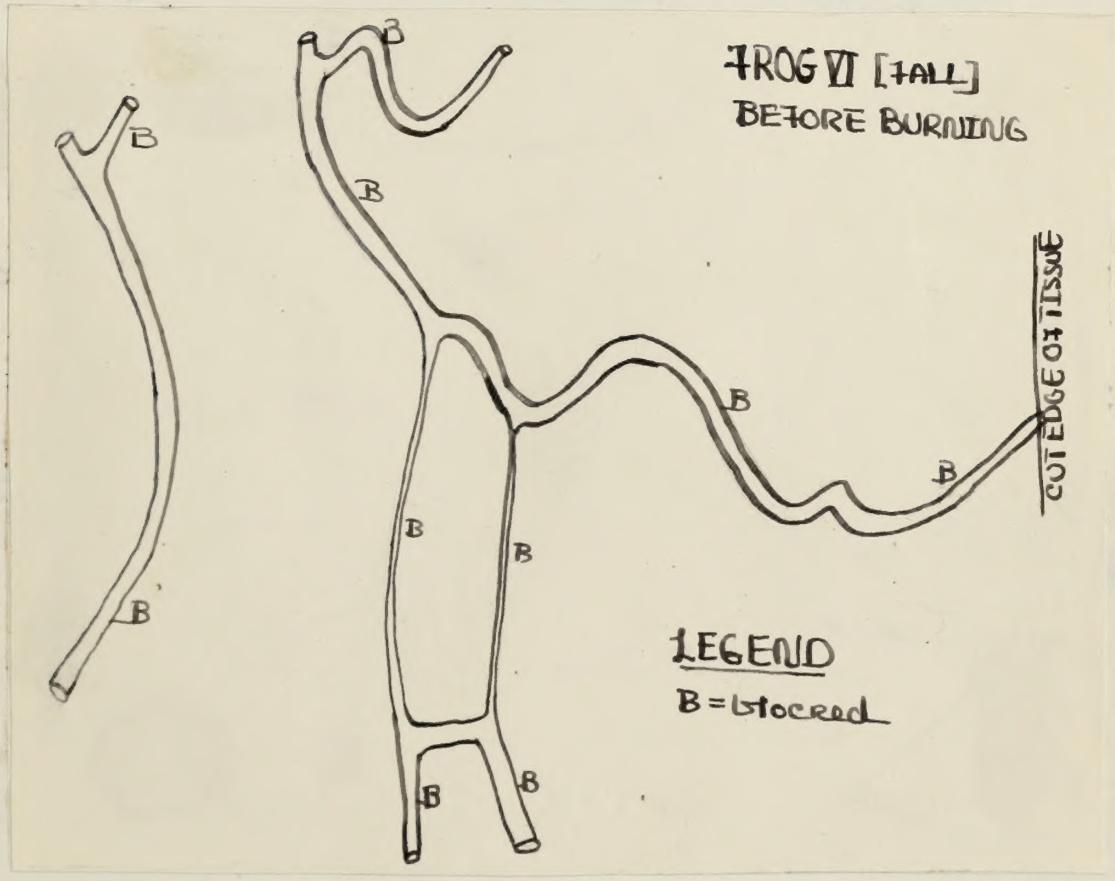

FROG VI [FALL]
BEFORE BURNING
B
B
B
B
B
B
B
B
B
B
CUT EDGE OF TISSUE
LEGEND
B = blocked

FIGURE IX

Figure X. Shows the three area effect present in sufficiently severe burns. It also illustrates the daily movement of area two and the fragmented appearance of the vessels in this area.

FROG VI

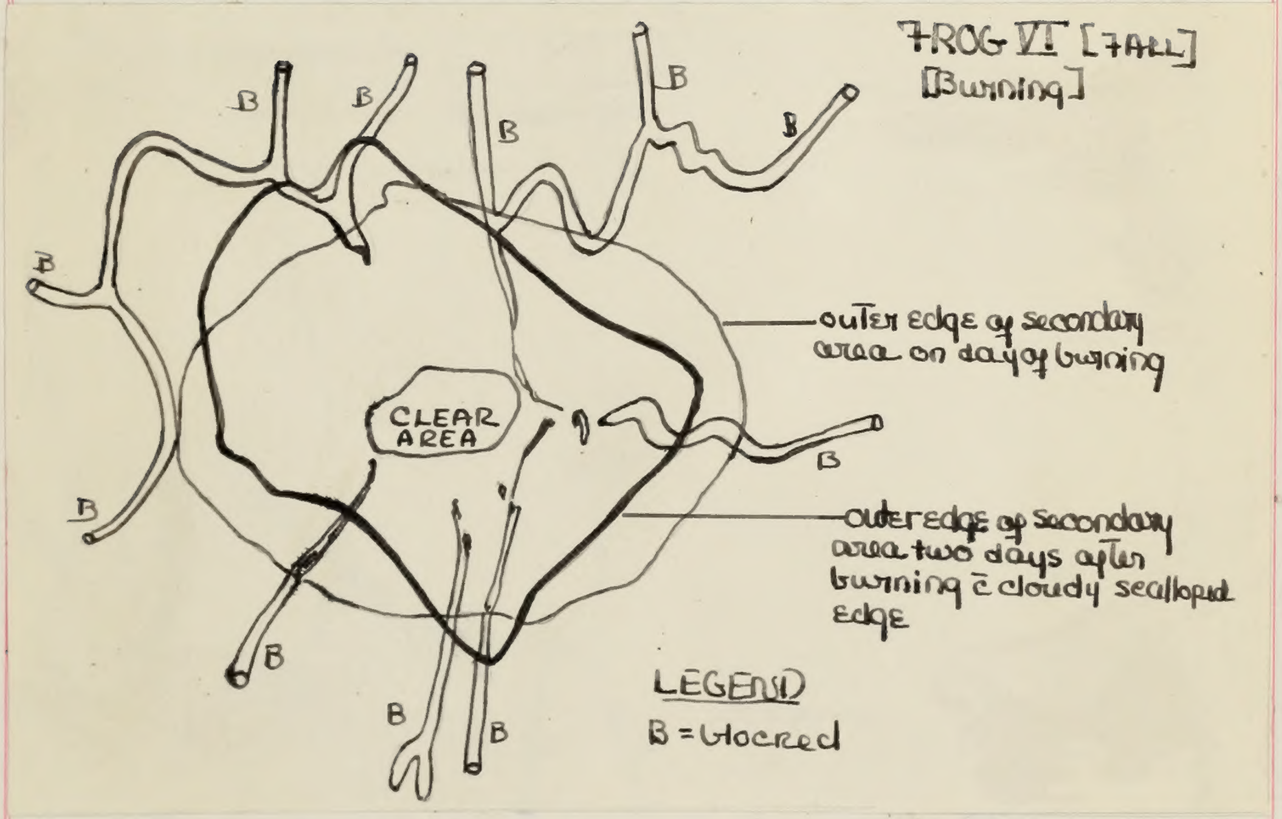

FROG VI [FALL]
[Burning]
B
B
B
B
B
B
B
CLEAR AREA
outer edge of secondary area on day of burning
B
outer edge of secondary area two days after burning c̄ cloudy scalloped edge
B
B
B
LEGEND
B = blocked

FIGURE X

Figure XI. Illustrates the usual pattern of degeneration of blood vessels and also the reestablishment of circulation in the nearby region. Area two has disappeared except for the band of cells at the lower left.

FROG VI

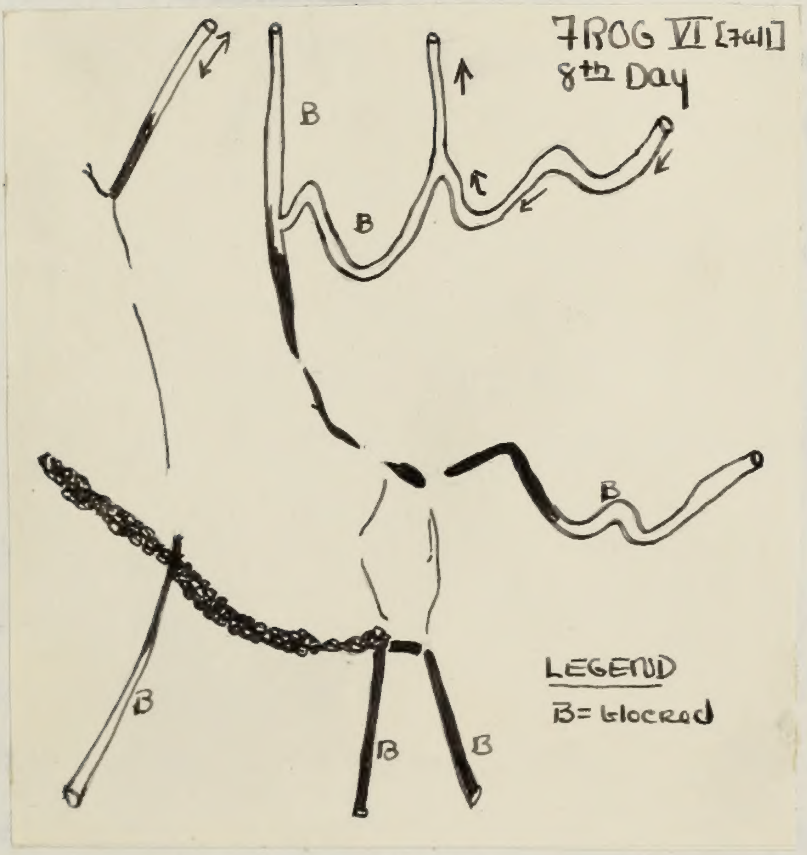


FIGURE XI

Figure 41.

FROG VII

June 15, 1946

9:00 .7 cc. urethane Dissection made; drawing made.

Before: Circulation is active.

Area chosen is clear.

After: Circulation has stopped; one arteriole remained irregularly contracted throughout.

Area burned:

1. There is a deposition of carbon particles (Figure XIIa).
2. There is no hole.
3. There is no three area effect.

June 17, 1946

9:00 .6 cc. urethane

9:15 .2 cc. "

9:40 .2 cc. "

Circulation is active around the burned area, but not in the area itself.

Area: The black debris has disappeared, but a hole is present (Figure XIIb) across which fibers are suspended. The fiber on the right side is a muscle fiber, for under high power the striations are quite marked (this was checked by Dr. Lutz). The other fibers appear to be

connective tissue.

Immediately around the hole are fractured muscle fibers, and above and below it are the severed ends of a once continuous capillary. To the right and above the hole are the severed ends of another capillary, each of which have extravasated erythrocytes at the tip, presenting the typical appearance of capillary sprouts (Figures IV, V, VI, and VII) with white cells in the nearby area. At 9:45, pulsation in the vessels is visible, but the pulsations never reach the vessel tips. Between the ends of this vessel are a few connective tissue fibers extending from the tip of II to III.

June 19, 1946

10:00	.6 cc urethane	The frog is reasonably lively; there is a small scar on rump, but not from the hypodermic. The skin has sloughed off the back at this spot.

II and III are joined, and there is marked repair. See diagram (Figure XIIc).

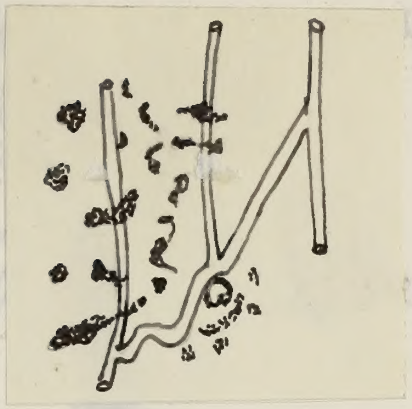
June 20, 1946

Frog VII is dead. The hole has not healed, and the rest of the membrane is much contracted.

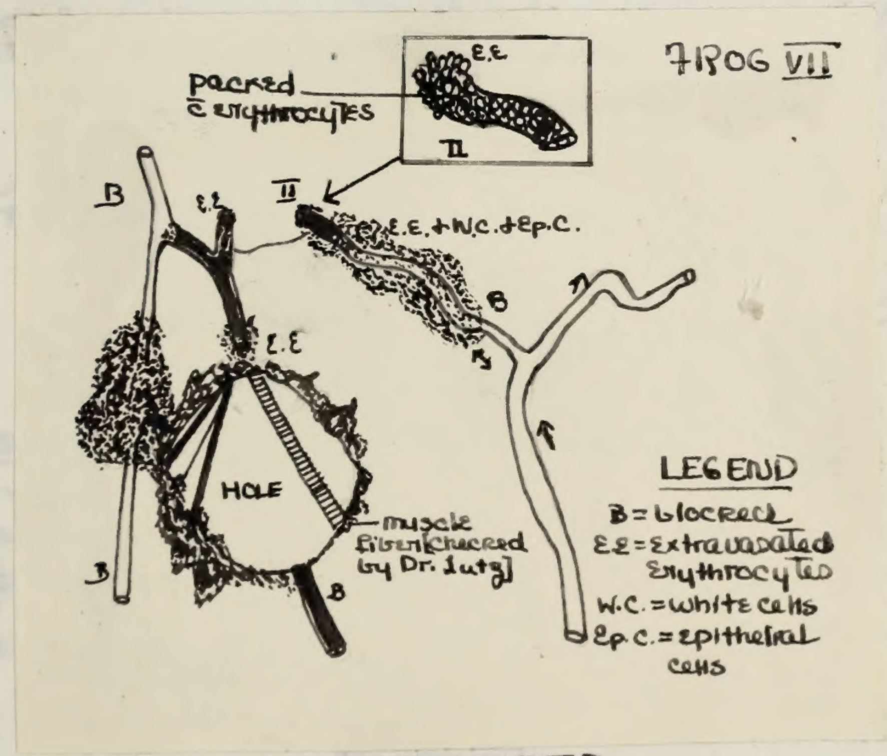
Figure XIIa. Illustrates the deposition of carbon granules following a burn with a dissecting needle and an alcohol lamp, and the cessation of circulation following a burn.

Figure XIIb. Illustrates regeneration of a muscle fiber and the typical capillary sprout appearance of the regenerating damaged vessel.

FROG VII



IMMEDIATELY AFTER BURNING
FIGURE XII a.



2nd DAY AFTER
FIGURE XII b

Figure XIIc: Illustrates final regeneration of the two capillaries and partial repair of the hole.

FROG VII

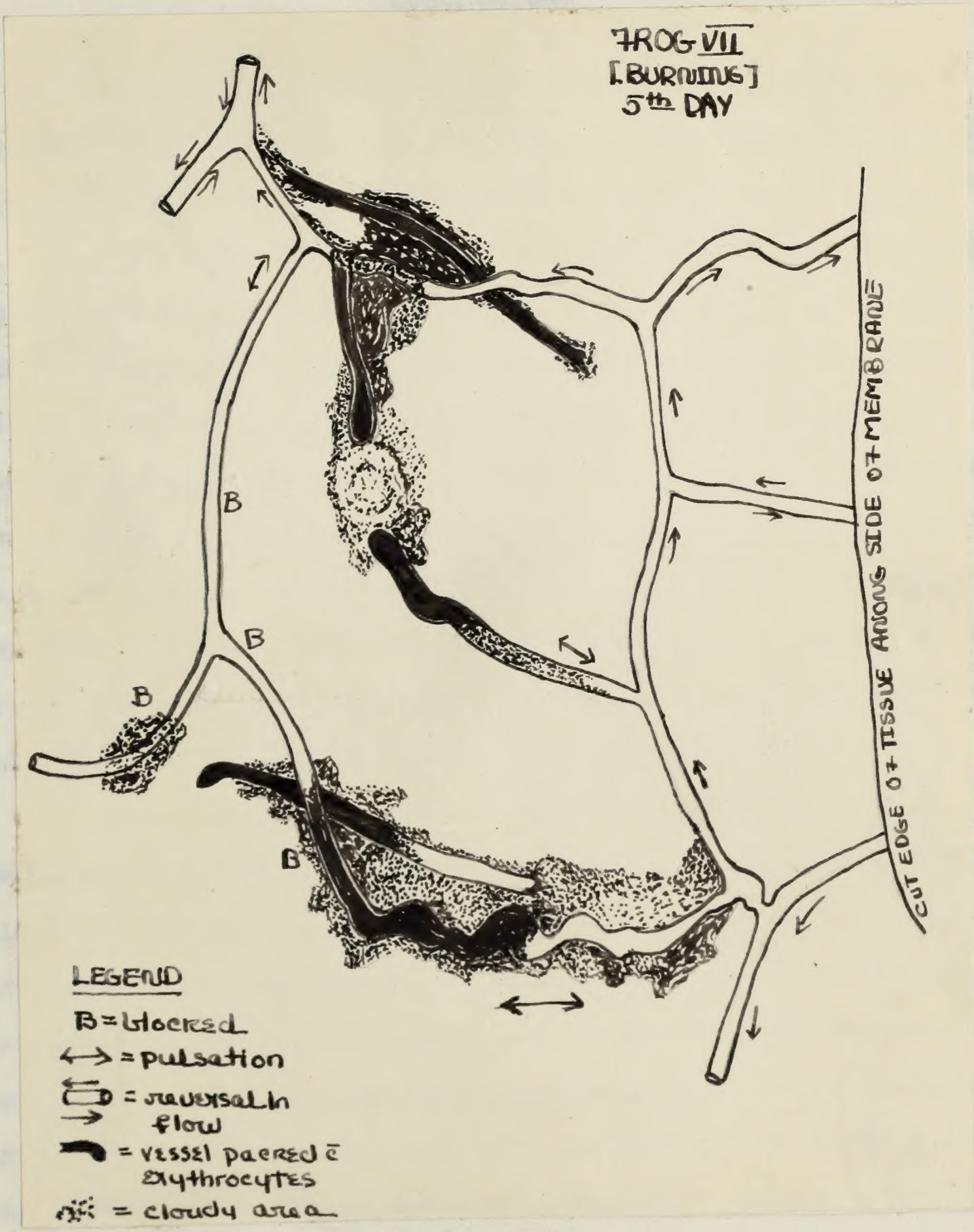


FIGURE XII c

CONCLUSIONS

The effect of burning on living tissues consists of destruction, inflammation, and healing, coupled with the modification of the last two processes by infection.

Destruction is a well established result accompanying a sufficiently severe burn; it includes all tissues which the burn affects, either by direct or conducted heat. Harkins (1945), in his paper The Present Status of the Problem of Thermal Burns, states that necrosis always results from a deep burn, owing to tissue destruction. Evidences of such destruction are coagulation of the tissues, edema due to increased capillary permeability resulting from damage to the walls, increased fragility of the erythrocytes, and necrosis, with the subsequent disintegration of the tissues.

Inflammation exhibits a definitive pattern of heat, swelling, and edema, which may be elicited not only by infections or irritating substances, but also directly by burns, as a result of heat damage to the capillary wall, which increases capillary permeability and causes loss of plasma from the vessel. In addition, a burn, acting indirectly through infection, may possibly cause the walling off of lymphatics, and produces a slow and somewhat irregular healing process. It is also to be noted that the same cellular sequence that is

present in inflammation is also present in burns.

The pattern and success of healing depend more on the extent and depth of injury than on the means of injury, as was indeed reported by Bishop (1945). The work discussed in this thesis (in "Observations", q.v.) further substantiates this conclusion. The main reason for slowness of healing in burns is probably that burns are more susceptible to infection than other types of wounds, as Dann, Glucksmann, and Tansley (1941) demonstrated.

ABSTRACT

The effect of burning on living tissues essentially involves initial destruction or injury of the tissues, subsequent inflammation and possible infection, and eventual healing. Destruction of tissues is a well-established result accompanying a sufficiently severe burn; it includes all tissues which the burn affects, either by direct or conducted heat. In scientific literature, evidence that a sufficiently severe burn causes tissue destruction has been presented by Best and McHenry (1931), Strassmann (1933), Bonciu (1935), Dann, Glucksmann, and Tansley (1941), Hughes and Dann (1941), Elman, Cox, Lischer, and Muller (1942), Leach, Peters, and Rossiter (1943), Evans and Hoover (1943), Prinzmetal, Hechter, Margoles, and Feigen (1944), Ham (1944, 1944a), Hara (1929), Shen and Haw (1943), McDonald, Levenson, Davidson, Tagnon, and Taylor (1944) and Harkins (1945). The latter, in his paper (1945), The Present Status of the Problem of Thermal Burns, states that necrosis always results from a deep burn owing to tissue destruction. Evidences of such destruction are coagulation of tissues, edema due to increased capillary permeability resulting from damage to the walls, increased fragility of the erythrocytes, and necrosis, with subsequent disintegration of the tissues.

Inflammation of tissues, according to the classic definition, produces heat, redness, and swelling of the area concerned, effects which depend upon dilation and increased permeability of the capillaries. Menkin (1940) also includes, as definitive elements of inflammation, the walling off of the lymphatics, and the migration of polymorphonuclear leucocytes, followed by macrophages, into the area. Evidence that dilation and increased capillary permeability is caused by burning was given by Underhill and co-workers (1930), Underhill (1930-1931), Best and McHenry (1931), Beard and Blalock (1931), McIver (1933), Clark and Clark (1933), Ely (1943), Netsky and Leiter (1943), Cope and Moore (1944) and Harkins (1945).

It also seems possible that a burn, indirectly through infection, may cause walling off of the lymphatics, and a slow and slightly irregular healing process, but leave unchanged the sequence of cellular response to inflammation seen in the appearance of polymorphonuclear leucocytes and macrophages. Evidence for the walling off of lymphatics in burns was given indirectly by Dann, Glucksmann, and Tansley (1941) and Harkins (1945); for the healing process, by Dann, Glucksmann, and Tansley (1941); for cellular sequence in any inflamed area, by Nakahara (1925), Clark, Clark, and Rex (1936), Menkin (1940), and Jones (1945); and for the cellular sequence in burns, by Menkin (1940).

Healing will take place in tissues injured by mechanical, chemical or thermal means, provided the injury is not so severe that grafting is necessitated. The papers of Sandison (1928, 1928a), Clark and Clark (1932)(1933)(1937), Arey (1932), Stearns (1940), and Dann, Glucksmann, and Tansley (1941) offer evidence of healing following mechanical injury to tissue. Regeneration of epithelial and connective tissues, blood vessels, lymphatic capillaries, and muscle fibers occurs. Dann, Glucksmann, and Tansley (1941), Hughes and Dann (1941), Glenn (1944), Patey and Scarff (1944), Converse and Roble-Smith (1944), Bishop (1945), Harkins (1945) and Strassmann (1933) offer evidence that epithelial and connective tissues, blood vessels, and muscle fibers regenerate following tissue injury by thermal or chemical means; whether nerves and lymphatics likewise regenerate has not yet been investigated.

Observations were made by the writer on the transilluminated retrolingual membrane of frogs, anesthetized either by urethane or animal nembutal. Control membranes were found to vary more in the appearance of blood vessels (thread-like capillaries and capillary sprouts) and less in the appearances of other tissues. Inflammation occurred, presenting the typical symptoms. Healing resulted in one of two ways: by the gradual encroachment over the cleared portion of the membrane of the tissues comprising the outer surface of the tongue, or

by the growth of tissues between the closely juxtaposed wound edges, free of the membrane itself, when the tongue was left undisturbed. In shock, thread-like capillaries appeared in profusion in the membrane and exhibited the common appearance of two "V"-shaped vessels linked by a connecting thread-like capillary.

Following experimental burning, tissue destruction was observed at first, with all tissue cells in the region having an aberrated, refractile appearance, the area consequently appearing cloudy and spotty; with a severe burn, the three area effect was observed, vessels were destroyed, and circulation ceased. Increased capillary permeability resulted, as evidenced by the leucocytes and the erythrocytes which appeared on the membrane in the burned area. This, plus the swelling, silting-up of the vessels, and the migration of ciliated epithelial cells in and near the area, provided the typical picture of inflammation. If infection occurred, inflammation was intensified and healing slowed. Regeneration occurred in blood vessels, connective tissues, and epithelial tissues, and was once observed in a muscle fiber. In the case of the blood vessels, both regeneration and degeneration were observed. In either case, extravasated erythrocytes were present around and at the tips of the vessels; these disappeared, however, from the portions of the degenerating vessels which had

become thread-like, and these thread-like portions would gradually break into smaller portions and finally disappear. Since no frog lived long enough for complete healing to occur, it was impossible to judge accurately how normal in appearance the regenerated epithelial and connective tissues were.

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